SPORE GERMINATION IN A MYXOMYCETE, *FULIGO SEPTICA* (L.) WEBER

by

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ABSTRACT

Spores of *Fuligo septica* (L.) Weber were studied by light and electron microscopy to determine structural changes during germination. Light microscopic observations indicate that few changes occur prior to protoplast release; however, electron microscopic observations show that a number of changes occur within the protoplast before emergence. An ovoid nucleus becomes irregular and lobed; smooth, cisternal endoplasmic reticulum develops; and concurrent development of dictyosomes and centriole occurs. The dictyosomes and centriole are localized in juxtanuclear sites, and the proximal cylinder of the centriole differentiates into a basal body of a future flagellum. When the spore case ruptures, the inner layer of the wall disappears and the nucleus reverts to its original ovoid form. The protoplast emerges through a wedge-shaped split in the wall and gradually develops into a flagellated cell or, sometimes, a myxamoeba. Simultaneously, contractile and food vacuoles develop, the cisternal endoplasmic reticulum becomes ribosome-coated, and a flagellum develops from the basal body.
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INTRODUCTION

Germination of Myxomycete spores was described first by de Bary in 1854. He observed the release of flagellated swarm cells from spores of Trichia rubiformis (now called Hemitrichia vesparium). Subsequent studies on spore germination indicated that the rate of germination, percentage of germination, and method of germination vary from species to species.

Most investigators studied the influence of external factors on spore germination in Myxomycetes. Smart (1937) investigated the effect of medium on germination. He found that the rate and percentage of spore germination of many Myxomycete species were increased if spores were sown in weak decoctions of natural substrata such as humus, rotted wood, and decayed leaves. Lister (1901) and Durand (1894) indicated that repeated wetting and drying of spores increased the rate and percentage of germination. Elliott (1949) noted that repeated wetting and drying had no effect on germination in some of the Myxomycetes. He emphasized that the germination rate increased when spores were wetted with a one percent bile salt solution. Other wetting agents used in attempting to increase the rate and percentage of germination were alcohol, detergents, mercuric chloride, and trisodium phosphate (Cayley, 1929; Elliott, 1949). Conflicting results were reported.

Temperature was shown to be an important external factor affecting both rate and percentage of germination as well as method of germination. Smart (1937) indicated that spore germination was best at
approximately 25°C to 30°C (see also Constantineanu, 1906), and that the rate and percentage of germination were reduced greatly if the temperature was below 10°C or above 30°C.

The influence of light, hydrogen ion concentration, spore concentration, and spore age are other factors which have been investigated. Light appears to have no influence on germination (Smart, 1937). The optimum pH for spore germination in most species is between pH 4.5 and pH 7.0 (Smart, 1937). Concentration of sown spores influences rate and percentage of germination (Scholes, 1962; Smart, 1937; Wilson and Cadman, 1928). Scholes (1962) has stated that spores germinate more rapidly and the percentage of germination is greater with dilute suspensions of spores; whereas, Smart (1937) and Wilson and Cadman (1928) have reported the opposite effect of spore concentration on germination. Spore age influences the rate and percentage of germination in some species (Alexopoulos, 1963).

Several types of spore germination have been described. In some Myxomycetes the protoplast escapes through a wedge-shaped crack in the spore wall (Gilbert, 1928; Howard, 1931; McManus, 1961; Smart, 1937). In other species the protoplast exits the spore case through an irregular pore (Gilbert, 1928; Smart, 1937). The protoplast, at the time of emergence, has been described as a myxamoeba or flagellated swarm cell, depending on the species and on environmental conditions (Gilbert, 1928; Smart, 1937). In some Myxomycetes the myxamoeba remains quiescent for a few minutes and then develops into a flagellated swarm cell (Howard, 1931; Smart, 1937). The swarm cell has been reported to
be uniflagellated or biflagellated, and in a few species, tri-
flagellated swarm cells have been observed (Yuasa, 1935, not seen -
see Elliott, 1949).

Few studies on cytoplasmic organelle changes during germination
of fungal spores have been published. Investigators have noted the
development and enlargement of a vacuole prior to germination (Gilbert,
1928; Smart, 1937). Concurrent with vacuolar formation is movement of
cytoplasmic granules. Gradually the spore wall becomes stretched and a
pore develops through which the protoplast emerges (Gilbert, 1928; Smart,
1937). Flagellar formation is reported to occur subsequent to pore or
crack development in the spore wall (Gilbert, 1928; Howard, 1931; McManus,
1961; Smart, 1937).

Ultrastructural changes in Myxomycete spores during germination
have not been reported, and few papers have been published on the fine
structure of Myxomycete spores (Loquin, 1959; Schuster, 1964; Wohlfarth-
Bottermann, 1959). The fine structure of myxamoebae has been observed
in Didymium nigripes (Schuster, 1964), and Cohen (1959) has studied
flagellation in some Myxomycete swarm cells.

Little is known about changes that occur from the time of spore
formation to the period in which swarm cells or myxamoebae are released.
Thus, the purpose of this study is to investigate the sequential
changes occurring in Myxomycete spores during germination. Fuligo septica
(L.) Weber, spores have been selected for this study because they
germinate rapidly and in high percentages in the laboratory.
MATERIALS AND METHODS

Spores of *Fuligo septica* were obtained from aethalia which had been collected on the University of British Columbia Endowment Lands and identified by R. J. Bandoni, July, 1963.

Approximately 0.1 g of spores was wetted in a one percent Difco bile salt solution for one minute and rinsed twice with sterile carbon-filtered, distilled water. The spores were sown in 60 x 20 mm sterile plastic disposable petri dishes which contained either 25 ml of sterile carbon-filtered, distilled water or 25 ml of sterile leaf-extract decoction. The leaves used in making the leaf-extract decoction were from deciduous trees and had been picked at random on the University of British Columbia Endowment Lands.

The percentage of germination and rate of germination were determined by averaging the results of duplicate spore suspensions subjected to the same conditions. To determine the percentage of germination, one drop of spore suspension was pipetted onto a glass slide, stained with iodine potassium iodide (IKI), and examined by light microscopy. The first 300 spores examined were used to determine the germination percentage. Approximately 90 to 95 % of the spores germinated 17 to 20 hours after they had been wetted in the bile salt solution and sown in either sterile carbon-filtered, distilled water or in leaf-extract decoction.

The optimum temperature for germination was determined by incubating the spore suspension in temperature controlled chambers set at approximately 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C. The temperature at which the rate of germination and percentage of germi-
nation was greatest was approximately 25°C. This temperature was designated as the optimum temperature for spore germination and further germination studies were performed at this established optimum temperature.

A light microscopic study of the germination process was performed with living material and material stained with IKI. Spores were examined with a Lietz Dialux microscope using bright field illumination, dark field illumination, and phase contrast. Micrographs were taken with a Lietz Orthomat microscope.

The material for electron microscopy was fixed at four different times during the germination period. The times arbitrarily were decided on and designated as stages one, two, three, and four. Stage one represented resting spores which were not subjected to germination treatment. Spores of stage two were wetted and sown in the germination medium for six hours, and those of stage three for twelve hours. Stage four represented the spores in which the protoplasts were about to emerge from the spore cases or which had emerged from the spore cases.

Fixation of material for electron microscopy was with unbuffered 1.5% KMnO$_4$ for 20 minutes at 0°C or 1% OsO$_4$ buffered with .15 M phosphate at pH 7.2 for one hour at 0°C. After the spores were washed well with distilled water, they were embedded in a drop of 4% water agar. The agar drop was cut into small pieces and the pieces were dehydrated in a graded series of ethanol-propylene oxide solutions. Modified Maraglas (Bisalputra and Weier, 1963) was used to embed the dehydrated material and the blocks were polymerized 18 to 24 hours in a vacuum oven at 65°C. The material was sectioned with glass knives with either a Porter-Blum MT 1 or MT 2 microtome. After mounting the sections on collodion-coated copper grids, the specimens were post-stained with uranyl acetate and lead citrate (Reynolds, 1963). All sections were examined with a Hitachi HU-11A microscope.
RESULTS

Approximately 90 to 95 % of the spores of *Fuligo septica* germinated after 17 to 20 hours in either sterile leaf-extract decoction or in sterile carbon-filtered, distilled water. Spores sown in sterile leaf-extract decoction tended to germinate slightly sooner than spores sown in sterile carbon-filtered, distilled water.

Light Microscopy

Few changes within the spore case during germination can be observed with the resolution of the light microscope. The spores appear spherical (Fig. 1), 6 to 9 μ in diameter, and the spore wall is spinulose. The protoplast, at first quiescent, becomes agitated; and a distinct nucleus is observed in iodine potassium iodide stained material. The spherical nucleus seems to maintain its form during germination. There appears to be a slight increase in the number of vacuoles within the protoplast prior to the splitting of the spore case.

Approximately 17 hours after wetting the spores, wedge-shaped splits develop in the spore cases. The splits gradually become more pronounced until their length equals at least three-fourths the diameters of the spores. Simultaneously, there is considerable movement of the protoplasm and the protoplasts begin to emerge from the spore cases (Figs. 2 - 5). The period of time for the emergence of the protoplasts is 3 to 5 minutes. Only one protoplast emerges from each spore.

When released, the protoplasts assume a spherical shape and remain in an oscillatory state near the mouths of the rupture for 10 to 15 minutes (Figs. 6 - 7). The protoplasts then become amoeboid and rapidly develop into flagellated swarm cells. No flagella are seen until after the protoplasts become amoeboid.

Most of the swarm cells are uniflagellate (Figs. 9 - 10); however, biflagellated cells are not uncommon (Fig. 8). The flagella of both uniflagellated and biflagellated cells are about the same length as the bodies of the swarm cells (Figs. 8 - 10).
Electron Microscopy

Stage One - Resting Spore

The typical structure of most resting spores observed in this study is seen in figure 11. A description of the ultrastructure of the resting spore follows:

Wall: A multi-layered, spinulose wall surrounds the spore protoplast (W, Fig. 11). It appears structurally similar to the spore walls of D. nigripes (Schuster, 1964). The outer layer of the wall (OW, Fig. 12) is electron dense, granular, and is approximately 50 \textmu m wide. Electron dense spines arise at irregular intervals on the surface of the outer layer (Fig. 11). The spine material also appears granular, and each spine is about 150 \textmu m long. A fibrillar, and almost electron transparent inner layer (IW, Fig. 12) lies between the electron dense outer layer and the plasma membrane. This inner layer is approximately the same width as the outer layer, and it is sub-divided into two sections by a 100 \text{\AA} electron dense line. The electron light section outside the electron dense line is approximately 40 \textmu m wide. The section adjacent to the plasma membrane varies in width, ranging from 2 \textmu m to 4 \textmu m. The electron dense line appears similar to the electron dense outer layer of the wall (Fig. 12).

Plasma Membrane: A distinct plasma membrane envelops the protoplasm of the spore (PM, Fig. 11). It is a 120 \text{\AA} thick, single unit membrane which is symmetrically 3 layered (45 \text{\AA} - 30 \text{\AA} - 45 \text{\AA}). The plasma membrane generally appears smooth; however, irregularities, as seen in figures 16 and 22, are not infrequent.
Vacuoles: There are several vacuoles in the cytoplasm of the spore (V, Fig. 11). These vacuoles are bound by single unit membranes and contain electron transparent or granular vacuolar sap; membrane-like fragments; and some electron dense, granular matter (Fig. 13). The vacuoles range in size from 1 to 2 μ.

Transparent Vesicles: Two types of electron transparent vesicles are dispersed randomly throughout the cytoplasm of the spore. One type of vesicle is membrane-bound and is about .1 to .3 μ in diameter (Fig. 11). Some of the membranes about these vesicles appear broken. The other type of electron transparent vesicle (TVe, Fig. 14) is not bound by a membrane. These vesicles are irregularly shaped and vary from .2 to .4 μ in diameter.

Mitochondria: Mitochondria with tubular-type cristae are distributed randomly within the protoplast (M, Fig. 11). The size, shape, and number of mitochondria per protoplast varies from spore to spore. Most mitochondria are somewhat spherical to ovoid and are about 1 to 1.5 μ in diameter (M, Fig. 15). Each mitochondrion is surrounded by an inner unit membrane and an outer unit membrane. The inner membrane, approximately 80 Å thick, invaginates forming tubular cristae which are about 25 μm in diameter. Each crista may branch several times forming side tubules. Most of the cristae extend generally towards the central region of the mitochondrion; however, a few of the cristae appear to be continuous with cristae arising from other regions of the mitochondrion (Fig. 15). The internal space of the tubular cristae is electron transparent, and the mitochondrial matrix is slightly more electron dense than the
cytoplasm (Fig. 15). An electron dense, coarsely granular matter occupies the central region of most of the mitochondria (Figs. 15 - 16). This dense core is about 100 μm in diameter and it appears to lack any organized structure (Fig. 16). The outer membrane surrounds the mitochondrion. This membrane does not invaginate and it is approximately as thick as the inner membrane. The mitochondrial membranes of the resting spores do not fix as distinctly as do the plasma membranes and the vacuolar membranes (Fig. 16).

Cytoplasm and Other Inclusions: Characteristic of the resting spore is a granular cytoplasm which is intermediate in density between the electron dense outer layer of the wall and the less electron dense vacuolar sap (Fig. 11). Some of the 100 to 130 Å granules in the cytoplasm resemble ribosomes in size and density (Fig. 14).

Nucleus: A single, ovoid nucleus is present in the protoplast of each spore of F. septica (N, Fig. 11). This nucleus, about 3 to 3.5 μ long and 2 μ wide, contains a single nucleolus (Nu, Fig. 17). The nucleolus is approximately 1 μ in diameter and is electron dense and granular. At least one nucleolar vacuole is present in each nucleolus, and neither the nucleolar vacuole nor the nucleolus is surrounded by a membrane. The chromatin material in the nucleus is almost as electron dense as the nucleolar granules, and it appears granular and randomly dispersed throughout the less electron dense nucleoplasm (Fig. 17). The nuclear material is encompassed by an envelope which is composed of two membranes, each about 80 Å thick. A perinuclear space, about 110 Å wide, is seen between the two membranes. Both the outer and the inner membranes of
the envelope are smooth, no ribosomes are present on their surfaces. Discontinuities in the nuclear envelope form "pores" which are 200 to 400 Å in diameter. These pores are simple and occur at irregular intervals (P, Figs. 17 and 20). No annuli are seen at the circumference of the pores and no diaphragms cross the diameter of the pores to separate the nuclear material from the cytoplasm. Bleb formation commonly is observed in the nuclear envelope (NB, Figs. 18 - 19). One type of bleb appears to be formed by the evagination of the outer membrane of the nuclear envelope and the formation of another membrane within this evagination (NB, Fig. 18). Thus, this type of bleb is encompassed by two membranes. A second type of bleb (NB, Fig. 19), surrounded by only one membrane, also is seen in a number of the nuclear envelopes. It appears to be formed by the evagination of the outer membrane and the invagination of the inner membrane of the envelope.

Stage Two - Six Hour Stage in Germination

Spores examined six hours subsequent to initiation of germination indicate few ultrastructural changes have occurred. These spores appear similar to the one shown in figure 22; however, vacuoles containing membrane-like fragments, not present in figure 22, are present in most spores at this stage.

Many spore organelles do not change in structure and, therefore, a description is made only when structural changes occur or when new organelles are encountered.

Nucleus: The nuclei of spores at this stage of germination assume a variety of forms (N, Figs. 22 - 23). The nuclear envelope becomes more
distinct and no bleb formation is distinguishable (Fig. 23). The chromatin material, nucleoplasm, and nucleolus do not appear to be altered (Fig. 23).

Mitochondria: Both the inner and the outer membranes of the mitochondria fix more distinctly than they do in the resting spore stage (Figs. 22 and 24), and the dark cores within the mitochondrial matrix become more prominent. Each core, approximately 100 to 150 μm in diameter, lies parallel to the longitudinal axis of the mitochondrion, and each is composed of numerous fine fibrils. Each fibril is about 30 Å in diameter. Some fibrils are seen in cross-section near the edges of the core, while other fibrils run parallel to the longitudinal axis of the mitochondrion (Fig. 24).

Transparent Vesicles: There is an increase in the number of small, membrane-bounded vesicles in the cytoplasm (Ve, Fig. 25). The membranes encompassing the vesicles are approximately 80 to 100 Å in thickness, while the diameter of the vesicles ranges from 50 to 100 μm. These vesicles are randomly distributed within the protoplast.

Endoplasmic Reticulum: Cisternal endoplasmic reticulum (ER, Fig. 25) is present in some of the spores. Each cisterna is a long, flattened sac-like structure which is bound by a unit membrane with an intracisternal space of about 100 to 120 Å in width. The bounding membrane is about 80 Å thick, and no ribosomes are present on the outer surface of this membrane (ER, Fig. 25).
Stage Three - Twelve Hour Stage in Germination

A number of changes occur within the spore protoplast within twelve hours after wetting the spores. Organelles not "detected" in the earlier stages of germination are observed, and some of the organelles described previously continue to change. The spinulose spore wall, plasma membrane, vacuoles containing membrane-like fragments, transparent vesicles, and membrane-bound vesicles remain unchanged (Fig. 26). The nucleus continues to change, centrioles and dictyosomes appear, and the cisternal endoplasmic reticulum becomes more extensive (ER, Fig. 27).

Nucleus: Most nuclei have become lobed (N, Fig. 27); however, the nuclear material appears to have changed little. A single nucleolus is present in each nucleus (Nu, Fig. 27).

Centriole: In some spores, a single centriole (Ce, Fig. 28) is seen in close proximity to the nucleus and in the central region of the protoplast. Each centriole is composed of a pair of cylinders, 400 to 430 μ long and 180 μ in diameter. The cylinders are open at both ends and lie at right angle to one another. Each cylinder is made up of nine longitudinal, evenly spaced, tubular fibrils (Ce, Fig. 29), and each fibril is about 350 Å in diameter and is composed of two subunits. At the end of the cylinder closest to the adjacent cylinder, the fibrils bend inward for about 300 Å at a 90° angle (Figs. 28 - 29). Two longitudinal, tubular fibrils lie in the central region of the cylinders, and each fibril is about 300 Å in diameter. In all
13.

Centrioles, there is cytoplasm of relatively low electron density in the central portion of the cylinder.

Dictyosomes: Most spores contain at least one or two dictyosomes, and each dictyosome is composed of a stack of three to four flattened cisternae with small vesicles at each end of the cisternae (D, Figs. 30 - 31). The cisternae resemble smooth-surfaced, cisternal-type endoplasmic reticulum. The membranes forming each cisternae are unit membranes, about 80 A° in thickness, and are separated by a low electron dense intracisternal space approximately 150 A° wide (D, Fig. 31). The dictyosomal cisternae lie parallel to each other and are separated by a space of about 120 A°. The small membrane-bound vesicles concentrated on each side of the stacks of flattened cisternae (Fig. 31) are about 200 to 400 A° in diameter.

Stage Four - Seventeen Hour Stage in Germination

There are four main developmental forms of *F. septica* spore protoplasts seventeen hours after wetting. Some protoplasts still are contained within the spore case (Fig. 32), others are emerging through splits in spore cases (Fig. 38), and many of the protoplasts have developed into swarm cells (Fig. 39) or myxamoebae (Fig. 40).

A. Spores Prior to Protoplast Emergence

When emergence of the protoplast has not occurred, both the inner and outer layers of the wall remain distinct. There is no indication of dissolution or cracking of any part of the wall (W, Fig. 32). Many nuclei still are lobed and sections through these lobes may give the impression
that more than one nucleus is present in a single spore (N, Fig. 32). However, in certain spores, the nuclei are less irregular and they assume a more oval pattern (N, Fig. 33-A).

Centrioles: Changes in centriole structure are seen prior to protoplast emergence. The centrioles assume the form of basal bodies with several types of rootlets extending from them (BB, Figs. 33 - 35). The nine outer fibrils are rotated evenly in the same direction and to a slight degree about a central fibril. Each outer fibril is connected to the adjacent fibrils and to the central fibril by fine fiber-like extensions, thereby, forming a "pin-wheel" pattern (BB, Figs. 33-B - 35). The outer fibrils are about 350 Å in diameter, the central fibril 450 Å in diameter, and the fiber-like extensions are about 40 Å wide. One form of rootlet tapers from the outer fibrillar region of the proximal cylinder and into the cytoplasm for about 100 to 150 μ (Fig. 33-B). The rootlets appear to be made of several tubules which are about 150 Å in width.

Another form of rootlet is seen in figures 34 and 35. A sheath of longitudinal fibrils partially surrounds the proximal cylinder of the centriole. These sheathing rootlets are about 300 μ long and 30 μ in diameter, and they appear to be connected to part of the wall of the proximal cylinder by fine, radiating fibers about 30 to 40 Å in width (Fig. 35). The sheath-like rootlets do not lie parallel to the proximal cylinder but seem to extend into the cytoplasm at an obtuse angle (R, Fig. 34). The distal cylinder of the centriole lies perpendicular to the proximal cylinder (Fig. 35).
Mitochondria: An electron dense, structured core is no longer visible in the central region of the mitochondria; however, the area formerly occupied by the core now appears less electron dense than the surrounding matrix (M, Fig. 36).

Vacuoles: Many vacuoles containing membrane remnants are surrounded by cisternal endoplasmic reticulum. This endoplasmic reticulum is not coated with ribosomes (Fig. 37).

B. Protoplast Emergence

In the second form, the protoplasts are seen emerging through splits in the spore walls (Fig. 38). Only the spinulose, electron dense, outer layer of the spore wall remains (Figs. 41 - 42). The inner layer, composed of two electron transparent sections separated by a dense line, is no longer visible. The nucleus reverts to an ovoid form and is in the cytoplasm which first exits the spore case. Small vacuoles are scattered throughout cytoplasm and the other organelles are not visibly altered. This freed protoplast then develops into either a flagellated cell (Fig. 39) or a myxamoeba (Fig. 40).

C. Swarm Cells and Myxamoebae

The swarm cell is pyriform in shape while the myxamoeba is of indefinite form. The myxamoeba and the swarm cell are structurally similar and both are bound by a single unit membrane, the plasma membrane (PM, Figs. 39 - 40). Both possess a single ovoid nucleus (N), a contractile vacuole (CV), mitochondria with tubular-type cristae (M), rough cisternal endoplasmic reticulum (RER), food vacuoles (V), dictyosomes (D), and
membrane-bound vesicles (Ve) (Figs. 39 - 51). In swarm cells, microtubules (MT, Fig. 39) pass about the nucleus in the cytoplasm and extend towards the basal body of the flagellum.

Endoplasmic Reticulum: Cisternal endoplasmic reticulum with ribosomes on its surface is present in all protoplasts that have emerged from spore cases. Each membrane is about 80 Å thick, and each intracisternal space is approximately 150 Å in width. Ribosomes are seen on the outer surfaces of the cisternae and are about 100 Å in diameter (RER, Fig. 43).

Food Vacuoles: Several vacuoles containing food particles are dispersed randomly throughout the cytoplasm of the myxamoebae and swarm cells. Each vacuole is bound by a single unit membrane, about 75 to 80 Å thick. The vacuoles range from .7 to 2 μ in size and contain a dense, granular, membrane-bound material. These granular, membrane-encompassed particles are suspended in an electron transparent vacuolar sap (V, Fig. 44). They vary in size, from .2 to .5 μ in diameter, and in shape.

Contractile Vacuole: Usually one contractile vacuole is present in each cell (CV, Figs. 39 - 40). In swarm cells, the contractile vacuole generally is situated in the posterior of the cell, or near dictyosomes; in myxamoebae, it does not appear to be located in any specific region of the cell. Contractile vacuoles vary in size and shape, depending on whether they are in an expanded state (CV, Fig. 45) or in a contracted state (CV, Fig. 46). Bordering contracted vacuoles are many small vesicles ranging from 40 to 70 μ in diameter. The membranes surrounding
both contractile vacuoles and bordering vesicles are about 80 Å thick.

Dictyosomes: Dictyosomes are found in most myxamoebae and flagellated cells. They are located usually in close proximity to the contractile vacuoles. In swarm cells, the dictyosomes are in the anterior of the cells between the basal bodies of flagella and the nuclei. Many dictyosome-like vesicles are visible in the anterior of the cells (Figs. 38 and 46). Structurally, the dictyosomes in swarm cells and myxamoebae appear more organized and defined than they do in protoplasts of stage three.

Flagella: Most swarm cells are uniflagellated; however, biflagellated cells are not uncommon. Each flagellum is 6 to 9 μ long, about 230 μm in diameter, and each is located in the anterior of the cell (Fig. 39). In cross-section, the flagellar structure is seen to be made of nine peripheral fibrils evenly spaced about two central fibrils (Fig. 50). Secondary elements are present between the two central fibrils and the nine peripheral fibrils. The peripheral fibrils are composed of two sub-units, each about 140 Å in diameter. On some of the peripheral fibrils, two arms appear to radiate out from one of the two sub-units. Each arm seems to be about 50 Å thick and approximately 70 Å long. The central fibrils lack sub-unit structure and are about 170 Å in diameter. The distance between the two central fibrils and the nine peripheral fibrils is about 350 Å, and the distance between the peripheral fibrils and the unit membrane which surrounds the axial filament is about 200 Å. The peripheral fibrils are about 120 Å apart and there appears to be a space of approximately 50 Å between the two central fibrils. The membrane about the axial filament is approximately 90 Å thick.
The basal bodies of flagella are sub-dermal and structurally similar to the basal bodies previously described in this study. In cross-section, each basal body is composed of nine peripheral fibrils about two central fibrils. Two sub-units comprise each of the outer fibrils. The dimensions of the sub-structure of the basal bodies is similar to that of the flagellum. Rootlets, which appear to be composed of linear aggregates of fibrils, extend from the periphery of the basal body into the cytoplasm (R, Fig. 47). Each fibril in the rootlet is about 120 \( \text{A}^0 \) in diameter. No fibrillar structure is visible in other rootlets (R, Fig. 48). These rootlets, 110 \( \mu \text{m} \) wide near the base of the flagellum, gradually taper and extend posteriorly in the cell. Microtubules (MT, Fig. 47) also are dispersed peripherally in the anterior of the swarm cell. They appear to terminate near the basal body of the flagellum. In figure 47, there are indications that some of the microtubules terminate in linear aggregates near the flagellar base.

Nucleus: Each swarm cell and myxamoeba possesses a single, ovoid nucleus (N, Figs. 39 - 40). The nucleus is located in the anterior third of the swarm cell near the flagellar basal body. No direct connection to the basal body from the nucleus has been observed. In the myxamoeba, the nucleus is not associated with a specific region of the cell. Each ovoid nucleus contains a single, electron dense, granular nucleolus which is not bound by a membrane and which has one to two nucleolar vacuoles (Nu, Figs. 52 - 53). The nucleolar vacuoles are electron transparent and are not encompassed by a membrane. Chromatin material is scattered randomly throughout an electron transparent nucleoplasm and it appears as clusters of electron dense granules (Figs. 51 and 54). A nuclear envelope surrounds the nuclear material. No bleb formation of this
envelope is seen and few pores are visible in the membranes of the envelope. The membranes forming the envelope are about 80 Å thick and the perinuclear space between the two membranes is about 130 Å wide (NE, Fig. 54).

**DISCUSSION**

*Fuligo septica* spores germinate readily in carbon-filtered, distilled water and in leaf-extract decoction subsequent to being wetted in a low percentage bile salt solution. Other investigators (Constantineanu, 1906, Gilbert, 1927; Scholes, 1962; Smart, 1937) report similar finding for *Fuligo septica* spores; however, a few investigators (Cook and Holt, 1928) indicate that a low percentage and a low rate of germination is obtained with spores of *F. septica* under similar conditions. Germination rates may differ even with two portions of a single collection of the same species, as has been observed in this study. Smart (1937) and others (Constantineanu, 1906; Wilson and Cadman, 1928; Scholes, 1962) have demonstrated that temperature, pH of the medium, age of spores, concentration of spores, and/or toxic substances in the medium inhibit or retard the rate of germination in many Myxomycete species. One or more of these factors may account for the poor results obtained by Cook and Holt (1928) and for the different rates of germination for two portions of the same collection.

The protoplasts of *F. septica* are freed from the spore cases in a manner similar to that described by Gilbert (1928) and others (Howard, 1931; McManus, 1961). Only the time required prior to the
splitting of the spore case appear to vary, with the way in which the spore case splits and the swarm cells develop being similar. After the development of a wedge-shaped split in the spore case does the protoplast emerge and assume a spherical form outside the mouth of the rupture. It remains there several minutes before it becomes amoeboid and develops into a swarm cell or myxamoeba. This resting period prior to swarm cell development is typical in several Myxomycete species (Elliott, 1949; Gilbert, 1928; Howard, 1931), and Kerr (1960) suggests that flagellar formation occurs at this time. Contrary to these observations, Smart (1937) states that for F. septica, "In those experiments of the writer in which the temperatures between 25°C and 31°C were used, the protoplasm of the spores always began to escape from the spore membrane immediately upon the formation of the aperature and gradually emerged. The split in the wall progressed as the protoplast escaped until the empty spore case showed a rupture equal to one-half or more the diameter of the spore. The protoplast then moved away from the empty spore membrane through amoeboid alterations of its form and within a few minutes become a flagellate swarm cell."

The swarm cells in this study seem to be mostly uniflagellate, but isokont biflagellate cells are not uncommon. Contrary to these findings, Elliott (1949) and Gilbert (1928) have reported that, in their germination studies, nearly all the swarm cells are heterokont biflagellates. Information on other Myxomycete species provides a partial explanation for these seeming contradictions. Cohen (1960 - see Alexopoulos, 1963) has demonstrated that the age of swarm cells,
condition of swarm cells, and species influences the percentage of bi-flagellate cells which form. Cohen (1959) and Koevenig (1961) (see Alexopoulos, 1963) both have shown that one or more pseudoflagella may be mistaken for true flagella. Kerr (1960) has observed in *Didymium nigripes* that uniflagellated cells develop first and that some of these cells may become biflagellated after several hours. Thus, discrepancies in results could be due to differences in *F. septica* strains, and/or the presence of pseudoflagella on uniflagellated cells.

A number of ultrastructural changes occur in *F. septica* during germination. The spores have multi-layered, spinulose walls which remain intact until the spores have ruptured to release the protoplasts. At this time, no electron transparent inner layer is visible and only the electron opaque, spinulose outer layer remains. The spore walls structurally resemble the spore walls of *D. nigripes* (Schuster, 1964; Wohlfarth-Bottermann, 1959). And, the spines of the wall appear to have been formed in a manner similar to that which Schuster (1964) describes for *D. nigripes* rather than as Cadman (1931-32) suggests. The latter author indicates that the protoplasm shrinks and causes the walls to buckle and form ridges on which spines develop. However, there is no space between the wall and the protoplast in *F. septica* spores which would allow for the collapse of the spore wall. The light area between the wall and the protoplast, which Cadman (1931-32) believes is a space, probably is an electron transparent inner layer of the wall similar to the one in spores of *F. septica*. How the inner layer breaks down
after the spore ruptures is not known, but several possible explanations can be suggested. The inner layer might rapidly dissolve in the medium once the spore ruptures, or the inner layer of the wall might be subject to enzymatic degradation prior to the splitting of the wall. Results of McManus (1961) tend to support the latter possibility. She has observed that in some germinating spores of *Clastoderma debaryanum*, one side of the spore case gradually becomes less dense and appears to dissolve. However, the inner layer of the walls remain visible in unruptured spores of *F. septica*.

The transparent vesicles which are not bound by membranes appear to be similar to those which have been described in the plasmodial and spore stages of *D. nigripes*, in plasmodia of *D. clavus*, *Stemonitis fusca*, *Hemitrichia vesparium*, *Clastoderma debaryanum* (Mc Manus, 1965; Schuster, 1964; Wohlfarth-Bottermann, 1959), and in many Eumycota (Blondel and Turian, 1960; Hawker and Abbott, 1963a, 1963b; Thyagarajan, Conti, and Naylor, 1961, 1962). McManus (1965) states that the transparent vesicles resemble secretory granules as found in animal gland cells, and Schuster (1964) believes that they have some type of contractile vacuolar activity. However, most of these vesicles closely resemble saturated and unsaturated lipid droplets which are dispersed throughout the cytoplasm. The lipid droplets then could: "serve as a local store of energy and a potential source of short carbon chains that can be used by the cell in the synthesis of its lipid-containing structural components, such as membranes, or in the elaboration of specific secretory products" (Fawcett, 1966).
Vacuoles containing membrane-like fragments and electron dense granular matter in the cell sap change little prior to swarm cell and myxamoeba development. However, once swarm cells or myxamoebae are formed, these vacuoles disappear. Similar appearing organelles have been described in other Myxomycetes as food vacuoles containing bacterial carcasses in varying degrees of digestion (Schuster, 1964; Wohlfarth-Botterman, 1959). These vacuoles also resemble food vacuoles in some Protozoa (Elliott and Clemmons, 1966; Mercer, 1959; Schuster, 1963). Lindegren (1962) states that yeast cells also possess vacuoles which contain membranes. However, he believes that these vacuoles are not residual food vacuoles but membrane synthesizing vacuoles. The synthesized membranes then are extruded into the cytoplasm forming endoplasmic reticulum of the cell.

Concurrent with the disappearance of the membrane-containing vacuoles is the appearance of definite food vacuoles (Fig. 44). The membrane-bound granular matter in these vacuoles structurally resembles bacterial cell protoplasm (Leene and van Iterson, 1965; Schuster, 1963). Thus, it seems that once the protoplast exits the spore case, it begins to ingest bacteria as a food source.

Contractile vacuoles develop at about the same time as food vacuoles, and usually one contractile vacuole is present in each swarm cell or myxamoeba. These vacuoles structurally are similar to contractile vacuoles of some Protozoa (Trager, 1964), and they probably function to eliminate excess water in a comparable manner. The small vesicles about the systolic vacuole suggest that rupturing of the tonoplast has occurred.
When the tonoplast fragments, many small vesicles are formed and the excess water and soluble wastes are eliminated from the cell. Soon after the vacuole discharges its contents, the small vesicles fuse and excess water and waste products diffuse into the vacuole until the pressure becomes too great and it again contracts. No nephridial tubules or endoplasmic reticulum appear to transport soluble wastes to the contractile vacuole as Elliott and Bak (1964) state occurs in the ciliate, *Tetrahymena pyriformis*.

Alterations in the endoplasmic reticulum during germination seem to be correlated closely with the changes in the physiological activity of the cells: the more active the cell, the more developed the endoplasmic reticulum. No cisternal or tubular endoplasmic reticulum is present in the resting spore; only smooth-surfaced vesicles are visible in the ribosome-dispersed cytoplasm of these cells. Whether the smooth surfaced vesicles actually are vesicular endoplasmic reticulum is not known. Unlike the motile cells of some Phycomycetes which form no endoplasmic reticulum (Cantino, et al., 1963), the protoplasts of *F. septica* develop smooth surfaced, cisternal endoplasmic reticulum. Later, when the swarm cells and myxamoebae form, the endoplasmic reticulum becomes ribosome-coated and appears similar to the endoplasmic reticulum which Blondel and Turian (1960) describe in *Allomyces macrogynus* cells. Thus, it seems that in *F. septica* the formation of ribosome-coated endoplasmic reticulum is associated with the more active state of the protoplast. The development of ribosome-coated endoplasmic reticulum in swarm cells of *F. septica* does not appear to be the same as it is
in yeasts. No rough endoplasmic reticulum is visible in nuclear associated vacuoles, and no rough endoplasmic reticulum is seen being extruded into the cytoplasm from these vacuoles, as Lindegren (1962) describes occurring in yeast cells. The ribosomes in the cytoplasm prior to swarm cell development appear to become attached to the cisternal endoplasmic reticulum to form ribosome-coated endoplasmic reticulum.

Dictyosomes, which are not visible in the resting spores of F. septica, appear to develop concurrently with the endoplasmic reticulum. Thus, the most organized form of the dictyosomes are seen in the swarm cells and myxamoebae. From these observations, and from observations of others (Fuller and Reichle, 1965; McManus, 1965), it appears that both the dictyosomes and the endoplasmic reticulum can be broken down and re-synthesized during the life-cycle of the organism. The more metabolically active the protoplast, the more structurally organized is the organelle. The dictyosomes and endoplasmic reticulum probably are most active during the swarm cell and myxamoeba stage because they appear best developed at this time. The dictyosomes always maintain a juxtanuclear position during germination. This relationship also is observed in the Eumycota (Fuller and Reichle, 1965; Hawker, 1963; Moore and McAlear, 1963), and it suggests that some type of control might be exerted by the nucleus. However, McManus (1965) reports that in plasmodia of several Myxomycetes, the dictyosomes are not localized in juxtanuclear sites but are scattered throughout the cytoplasm. In swarm cells the dictyosomes probably function in the secretory processes of the cell as they do
in other organisms (Dalton, 1961; Fawcett, 1966). In some Protozoa, the dictyosomes and contractile vacuoles are in close proximity to each other and share water removal activities in the cell (Cohn, 1964). A similar spatial relationship between the dictyosomes and contractile vacuoles exists in swarm cells of \textit{F. septica}. These organelles might function in a manner comparable to the dictyosomes in Protozoa.

The mitochondria structurally are similar to those in other Myxomycetes (Dugas and Bath, 1962; McManus, 1965; Schuster, 1965; Wohlfarth-Bottermann, 1959). However, they are unlike those of most other fungi. The mitochondria in Hemiascomycetes (Bandoni, Bisalputra, Bisalputra, in press; Lindegren, 1962; Thyagarajan, et. al., 1961), Euascomycetes (Moore and McAlear, 1962, 1963b; Shatkin and Tatum, 1959), some Phycomycetes (Hawker and Abbott, 1963a; Moore and McAlear, 1963b; Cantino, et. al., 1963), Basidiomycetes (Bandoni, personal communication; Moore and McAlear, 1963b), and Fungi Imperfecti (Hawker and Hendy 1963; Thyagarajan, et. al., 1962, 1963; Wells, 1964) all have lamellar-type cristae mitochondria. Only a few fungi other than Myxomycetes possess mitochondria with tubular-type cristae (Hawker and Abbott, 1963b; Fuller and Reichle, 1965), and in these forms, the tubules do not branch as they do in the mitochondria of \textit{F. septica} protoplasts and other Myxomycete plasmodia. Also, the motile cells of \textit{F. septica} possess numerous mitochondria, whereas, the motile cells of some fungi possess only a single mitochondrion (Cantino, et. al., 1963). The electron dense cores which are visible in the early stages of germination are probably nucleic acid cores which have been shown to be present in some
Myxomycetes (Schuster, 1965). The different appearance of these cores during germination may be a result of physiological alterations occurring within the cell. Such changes might influence, directly or indirectly, the reaction of the cores to the fixative.

Little is known about flagellar structure and development in Myxomycetes as few cytological studies have been made. Ross (1957) and others (Howard, 1931; McManus, 1961; Smart, 1937) show that either uniflagellated or biflagellated cells develop after the protoplasts have been freed from spore cases. Kerr (1960) and Ross (1957) also have demonstrated that each flagellum is anchored in the cell by a basal body. Electron micrographs of *F. septica* swarm cells show that the flagella have the typical 9 + 2 fibrillar arrangement which characterizes motile processes in other fungi (Fuller and Reichle, 1965; Kock, 1956, Cantino, et. al., 1963; Renaud and Swift, 1964) and other organisms (Fawcett, 1966). These flagella arise from basal bodies which may or may not be similar to basal bodies in other fungi. Of the few flagellated fungal cells which have been examined by electron microscopy (Fuller and Reichle, 1965; Cantino, et. al., 1963; Renaud and Swift, 1964), the structure of the basal body is similar but not identical. However, unlike some fungal (Berlin and Bowen, 1964) and animal (Fawcett, 1966) centrioles and basal bodies, in which each of the nine peripheral fibrils is composed of three sub-units, the nine peripheral fibrils of the basal bodies and centrioles in *F. septica* are made up of two sub-units. As in other fungi (Renaud and Swift, 1964), the basal body develops from the proximal cylinder of the
centriole which is in a juxtanuclear position. However, unlike other fungi (Renaud and Swift, 1964; Fuller and Reichle, 1965), a close association of the nuclei and basal bodies of *F. septica* swarm cells is not maintained during or after flagellar development. There is no indication of a direct connection by means of a rhizoplast between the two organelles, only dictyosome-like vesicles appear between the basal body and the nucleus. The function of these dictyosome-like vesicles is unknown; however, some investigators suggest that the vesicles fuse and form the flagellar sheath (Sorokin, 1962). The function of the rootlets extending from the basal bodies is unknown, but it is probably that they function in support and anchorage of the flagella. A similar function may exist for the microtubules which radiate about the base of the flagellum.

The nucleus undergoes a series of changes during germination and a few of these changes have been reported in other Myxomycetes. The ovoid nucleus, which is characteristic of the resting spore, resembles the nuclei Dugas and Bath (1962) describe in *Physarum polycephalum* plasmodia. However, while nuclei of *P. polycephalum* usually contain as many as four nucleoli, those of *F. septica* possess only a single nucleolus. Blebs, which may or may not contain nuclear material, are seen in the nuclear envelope of only the resting spores. These nucleocytoplasmic blebs structurally are similar to those in other organisms (Schuster, 1963; Gay, 1956). If these blebs contain nuclear material, as has been suggested by Gay (1956), then they might be able to direct certain cytoplasmic reactions and syntheses if the blebs should pass
into the cytoplasm. The pores in the nuclear envelope also allow for a certain amount of nucleo-cytoplasmic exchange. Schuster (1963) states that Porter (1960) has suggested that such particles as ribose nucleic acid (RNA) might pass through these pores into the cytoplasm. The extreme plasticity of the nucleus, as seen in *F. septica* spores during germination and in protoplasts of other Myxomycetes (Dugas and Bath, 1962; McManus, 1965; Locquin, 1949), indicates that there is interaction between the nucleus and the cytoplasm. The more irregular and lobed the nucleus, the greater is the surface for these interactions to occur. Thus, it appears that the greatest amount of interaction might occur during germination and not in the resting spore and swarm cell or myxamoeba stages.

The results of this study indicate that Myxomycetes, such as *F. septica*, are not closely related to true fungi. They structurally appear more closely related to Protozoa. Similarities in organelle structure tend to support this idea. Myxomycetes and most Protozoa possess mitochondria with tubular-type cristae, contractile vacuoles, food vacuoles, and similar dictyosome organization. The feeding habits of the two groups are similar, and neither group possesses a wall during most of their life-cycle. However, the presence of a wall during the spore stage and the centriole and basal body sub-structure more closely resemble characteristics of the lower plants and many fungi.
SUMMARY

Approximately 90 to 95% of the spores of *F. septica* germinate after 17 to 20 hours in either sterile leaf-extract decoction or in sterile carbon-filtered, distilled water following wetting in a bile salt solution. Spores which have been sown in leaf-extract decoction germinate slightly sooner than spores which have been sown in carbon-filtered, distilled water.

An ovoid nucleus containing a single nucleolus, mitochondria with tubular-type cristae, vacuoles with membrane-like fragments and electron dense material, lipid droplets, and membrane-bound vesicles characterize the resting spore protoplast. Each protoplast is surrounded by a multi-layered, spinulose spore wall.

Six hours after wetting the spores, the nucleus is very irregular in shape and many small vesicles are found in the cytoplasm. Small amounts of smooth cisternal endoplasmic reticulum also appear in the cytoplasm.

Before the spore ruptures to release their protoplasts, centrioles and dictyosomes develop concurrently in juxtanuclear sites. Also, the nucleus becomes lobed; and more smooth, cisternal endoplasmic reticulum develops. The proximal cylinders of the centrioles develop into basal bodies shortly before the splitting of the spore cases.

With the rupturing of the spore case, the inner layer of the wall disappears. Simultaneously, the protoplasts escape. They rest
a short time near the mouth of the rupture and then develop into flagellated swarm cells or myxamoebae.

In both swarm cells and myxamoebae, the endoplasmic reticulum becomes ribosome-coated, contractile-vacuoles and food vacuoles develop, and the nucleus is reverted to its ovoid form. Flagella attached to basal bodies also develop in the swarm cell. Microtubules are seen to radiate posteriorly from the basal body region of the flagella and probably give more rigidity to the motile cells.


APPENDIX
Fig. 1. *Fuligo septica* resting spore. X 1,800.

Figs. 2-5. Emergence of spore protoplast through wedge-shaped split in wall. X 1,900.

Fig. 6. Released protoplast assumes spherical shape outside mouth of rupture. X 1,900.
PLATE 2

Fig. 7.  Spherical protoplast in oscillatory state outside mouth of rupture.  X 1,900.

Fig. 8.  Biflagellated swarm cell.  X 1,600.

Fig. 9.  Uniflagellated swarm cell.  X 1,600.

Fig. 10.  Uniflagellated swarm cell.  Phase contrast.  X 1,150.
Fig. 11. Typical resting spore. Arrows within protoplast indicate small membrane-bound vesicles. W = wall, PM = plasma membrane, M = mitochondrion, TVe = transparent vesicle, V = vacuole, N = nuclear material, Nu = nucleolus. Phosphate buffered OsO$_4$ fixation. X 27,500.
PLATE 4

Fig. 12. Spinulose spore wall. OW = electron dense outer layer, IW = electron transparent inner layer, PM = plasma membrane. Phosphate buffered OsO₄ fixation. X 127,000.

Fig. 13. Vacuoles containing membrane-like fragments and electron dense granular matter in cell sap. Phosphate buffered OsO₄ fixation. X 37,150.

Fig. 14. Transparent vesicles. Note absence of membranes about each vesicle. Phosphate buffered OsO₄ fixation. X 42,000.
PLATE 5

Fig. 15. Mitochondria with tubular-type cristae in resting spore protoplast. Arrows indicate region where cristae branch forming other cristae within the mitochondrion. M = mitochondrion, TVe = transparent vesicle, V = vacuole. Phosphate buffered OsO₄ fixation. X 43,900.

Fig. 16. Mitochondrion with electron dense, granular core in the matrix. M = mitochondrion, PM = plasma membrane, IW = inner layer of wall, OW = outer layer of wall. Phosphate buffered OsO₄ fixation. X 127,700.
Fig. 17.  Nucleus.  NE = nuclear envelope, N = nuclear material, Nu = nucleolus.  Phosphate buffered OsO$_4$ fixation.  X 36,650.

Fig. 18.  Nuclear envelope showing one type of bleb commonly observed in the resting spore stage.  Note simple pore in envelope.  NB = nuclear bleb, N = nuclear material, P = pore.  Phosphate buffered OsO$_4$ fixation.  X 40,000.

Fig. 19.  Second type of nuclear bleb observed in the resting spore stage nucleus.  Note simple pores in envelope.  NB = nuclear bleb, P = pore.  Phosphate buffered OsO$_4$ fixation.  X 39,650.
Fig. 20.  Nucleus.  Note number of simple pores in envelope and membrane structure of envelope.  NE = nuclear envelope, P = pore, N = nuclear material, Nu = nucleolus.  KMnO₄ fixation.  X 37,500.

Fig. 21.  Section of the nuclear envelope showing the two unit membranes of the envelope.  Note the simple pores.  NE = nuclear envelope, P = pores, N = nuclear material.  KMnO₄ fixation.  X 65,000.
PLATE 8

Fig. 22. Cross-section of a spore six hours after wetting. Note nuclear shape and number of small vesicles in the cytoplasm. W = wall, M = mitochondrion, TVe = transparent vesicle, N = nuclear material. Phosphate buffered OsO₄ fixation. X 27,750.
Fig. 23. Nucleus six hours after wetting of spores. Note the irregular configuration of the nucleus. N = nuclear material, Nu = nucleolus. Phosphate buffered $\text{OsO}_4$ fixation. X 56,000.
Fig. 24. Mitochondrion of spore in stage two. Note fibrillar appearance of core. Phosphate buffered OsO₄ fixation. X 30,600.

Fig. 25. Cytoplasm of spore six hours after the beginning of germination. Note the number of small, membrane-bound vesicles and the small amount of cisternal endoplasmic reticulum. TVe = transparent vesicle, M = mitochondrion. Phosphate buffered OsO₄ fixation. X 49,375.
Fig. 26. Section through a spore twelve hours after wetting. The nucleus is not shown in this section. OW = outer layer of wall, IW = inner layer of wall, PM = plasma membrane, V = vacuole, M = mitochondrion. Phosphate buffered OsO₄ fixation. X 26,600.
Fig. 27. Lobed nucleus characteristic of spores twelve hours after the beginning of germination. Note cisternal endoplasmic reticulum about the nucleus. ER = endoplasmic reticulum, NE = nuclear envelope, N = nuclear material, Nu = nucleolus, M = mitochondrion. Phosphate buffered OsO₄ fixation. X 41,250.
Fig. 28. Centriole adjacent to nucleus. Cylinders of the centriole are open at both ends. N = nuclear material, Ce = centriole. Phosphate buffered OsO$_4$ fixation. X 72,850.

Fig. 29. Centriole at a greater magnification. Ce = centriole. Phosphate buffered OsO$_4$ fixation. X 133,575.
Fig. 30. Two dictyosomes in close proximity to nucleus. D = dictyosome, N = nuclear material, Nu = nucleolus. Phosphate buffered OsO$_4$ fixation. X 111,100.

Fig. 31. Dictyosome at higher magnification. Each dictyosome is composed of three to four cisternae lying parallel to one another. D = dictyosome, N = nuclear material. Phosphate buffered OsO$_4$ fixation. X 156,950.
Fig. 32. Spore prior to protoplast emergence. W = wall, PM = plasma membrane, TVe = transparent vesicle, M = mitochondrion, ER = endoplasmic reticulum, V = vacuole, N = nuclear material. Phosphate buffered OsO₄ fixation. X 41,400.
PLATE 16

Fig. 33-A. Section through a spore prior to protoplast emergence. Note that the basal body is present in the central region of the spore and in a juxtanuclear position. BB = basal body, N = nuclear material, TVe = transparent vesicle, M = mitochondrial, W = wall. Phosphate buffered OsO₄ fixation. X 22,000.

Fig. 33-B. Cross-section of a basal body at high magnification. Note that rootlets extend from periphery of basal body. BB = basal body, R = rootlets. Phosphate buffered OsO₄ fixation. X 85,550.

Fig. 34. Longitudinal section through proximal cylinder of a basal body. Note that a different type of rootlet than seen in figure 33-B extends into the cytoplasm from the basal body periphery. BB = basal body, R = rootlets. Phosphate buffered OsO₄ fixation. X 49,950.

Fig. 35. Cross-section through proximal cylinder of the basal body. Note that the rootlets radiate from the basal body and appear to be connected to the cylinder by fine fiber-like extensions. BB = basal body, R = rootlets. Phosphate buffered OsO₄ fixation. X 59,250.
Fig. 36. Mitochondrion typical of spores prior to protoplast emergence. Membranes of mitochondrion appear distinct and the "core" region less electron dense than the matrix. No fibrillar structure is visible in the core region. M = mitochondrion, V = vacuole. Phosphate buffered OsO$_4$ fixation. X 83,100.

Fig. 37. Smooth, cisternal endoplasmic reticulum usually is in close proximity to vacuoles containing membrane-like fragments. ER = endoplasmic reticulum, V = vacuole. Phosphate buffered OsO$_4$ fixation. X 79,200.
Fig. 38. Section through emerging protoplast. Note absence of inner layer of spore wall. The nucleus has reverted to ovoid shape and is in the cytoplasm which first exits the spore case. OW = outer layer of wall, M = mitochondrion, N = nuclear material. Phosphate buffered OsO₄ fixation. X 31,875.
Fig. 39. Longitudinal section through a swarm cell. The basal body of the flagellum is present in the anterior of the cell. Microtubules and rootlets radiate into the cytoplasm in the region of this basal body. A contractile vacuole is seen in the posterior of the flagellated cell, and ribosomes are visible on the surface of the endoplasmic reticulum. PM = plasma membrane, BB = basal body, MT = microtubules, N = nuclear material, M = mitochondrion, CV = contractile vacuole, RER = ribosome coated endoplasmic reticulum. Phosphate buffered OsO₄ fixation. X 33,750.
Fig. 40. Myxamoeba. Note the ovoid shape of the nucleus and the presence of a contractile vacuole. PM = plasma membrane, CV = contractile vacuole, M = mitochondrion, RER = ribosome-coated endoplasmic reticulum, N = nuclear material. Phosphate buffered OsO₄ fixation. X 36,800.
Fig. 41. Cross-section through spore walls. In upper part of micrograph, the protoplast still is retained within the spore case and the multi-layered wall appears to be completely intact. In the lower part of the micrograph, the spore case has ruptured releasing the protoplast and only the outer layer of the wall is visible. OW = outer layer of wall, IW = inner layer of wall. Phosphate buffered OsO₄ fixation. X 111,275.

Fig. 42. Spore wall after the protoplast has been released. Note that only the spinulose outer layer of the wall remains. OW = outer layer of wall. Phosphate buffered OsO₄ fixation. X 88,900.

Fig. 43. Ribosomes present on outer surface of cisternal endoplasmic reticulum. RER = ribosome-coated endoplasmic reticulum, M = mitochondrion, V = vacuole. Phosphate buffered OsO₄ fixation. X 41,150.
PLATE 22

Fig. 44. Vacuoles containing electron dense granular matter. The granular matter is bound by unit membranes. V = vacuole. Phosphate buffered OsO₄ fixation. X 106,350.

Fig. 45. Contractile vacuole in expanded state. CV = contractile vacuole, RER = ribosome-coated endoplasmic reticulum. Phosphate buffered OsO₄ fixation. X 63,475.
Fig. 46. Contractile vacuole in contracted state. Also, a dictyosome is seen in close proximity with the nucleus. N = nuclear material, D = dictyosome, Ve = vesicle, CV = contractile vacuole. Phosphate buffered OsO₄ fixation. X 108,250.
PLATE 24

Fig. 47. Cross-section through the basal body of the flagellum. The basal body is composed of nine peripheral fibrils about two central fibrils. Each peripheral fibril is made up of two sub-units. Extending from the peripheral area of the basal body are rootlets which are fibrillar-like in structure. Microtubules are seen radiating from the flagellar region posteriorly in the cell. PM = plasma membrane, MT = microtubules, BB = basal body, R = rootlets, Ve = vesicle. Phosphate buffered OsO₄ fixation. X 57,500.

Fig. 48. Longitudinal section through the anterior of a swarm cell. Rootlets and dictyosome-like vesicles are present in this area. R = rootlets, Ve = vesicle. Phosphate buffered OsO₄ fixation. X 33,700.

Fig. 49. Cross-section through a flagellum in a swarm cell. Note the 9 + 2 fibril arrangement. N = nuclear material, Nu = nucleolus. Phosphate buffered OsO₄ fixation. X 37,500.

Fig. 50. Cross-section through a flagellum. Note the 9 + 2 fibril arrangement and the secondary elements which are present between the nine peripheral fibrils and the two central fibrils. A unit membrane surrounds this axial process. Phosphate buffered OsO₄ fixation. X 82,650.
Typical nucleus of swarm cells and myxamoebae. The nuclear material is surrounded by a distinct nuclear envelope. A single nucleolus is present in each nucleus. NE = nuclear envelope, N = nuclear material, Nu = nucleolus. Phosphate buffered OsO₄ fixation. X 44,300.

Nucleoli with one or two nucleolar vacuoles. No membrane encompasses the nucleolar vacuoles. Nu = nucleolus. Phosphate buffered OsO₄ fixation. X 62,475.

Nuclear envelope at higher magnification. Note the two unit membranes forming the envelope. N = nuclear material, NE = nuclear envelope, RER = ribosome-coated endoplasmic reticulum. Phosphate buffered OsO₄ fixation. X 88,000.